

Listing of Claims:

This list of claims replaces all previous listings.

1-4. (Canceled)

5. (Withdrawn) An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;

(b) a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:2;

(c) a polynucleotide encoding a polypeptide that comprises an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 2 in which one or more amino acids are substituted, deleted, inserted, and/or added and that is functionally equivalent to a polypeptide comprising the amino acid sequence of SEQ ID NO:2; and

(d) a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1 and that encodes a polypeptide functionally equivalent to a polypeptide comprising the amino acid sequence of SEQ ID NO:2.

6. (Withdrawn) The isolated polynucleotide of claim 5, wherein the polynucleotide comprises a nucleotide sequence having 70% or higher percent identity to the nucleotide sequence of SEQ ID NO:1.

7. (Withdrawn) The isolated polynucleotide of claim 5, wherein the polynucleotide encodes an amino acid sequence having 70% or higher percent identity to the amino acid sequence of SEQ ID NO:2.

8-9. (Canceled)

10. (Withdrawn) A vector comprising the polynucleotide of claim 5.

11. (Withdrawn) A transformant comprising the polynucleotide of claim 5.
12. (Withdrawn) A transformant comprising the vector of claim 10.
13. (Withdrawn) A method for producing a polypeptide, the method comprising the steps of: culturing the transformant of claim 11 and recovering an expression product.
14. (Withdrawn) A method for producing an (R)-2,3-butanediol dehydrogenase, the method comprising: (a) culturing a microorganism that belongs to the genus *Pichia* and that produces the dehydrogenase of claim 1 and (b) isolating the dehydrogenase from the microorganism.
15. (Withdrawn) A method for producing an (R)-2,3-butanediol dehydrogenase, the method comprising: (a) culturing a microorganism that belongs to the genus *Pichia* and that produces the polypeptide of claim 8 and (b) isolating the dehydrogenase from the microorganism.
16. (Withdrawn) The method of claim 14, wherein the microorganism is *Pichia angusta*.
17. (Withdrawn) A method for producing an alcohol, the method comprising the steps of:
reacting the (R)-2,3-butanediol dehydrogenase of claim 1 or a processed product thereof to a ketone in the presence of reduced form of nicotinamide adenine dinucleotide to generate an alcohol, and
recovering the generated alcohol.

18. (Withdrawn) A method for producing an alcohol, the method comprising the steps of:

reacting the polypeptide of claim 8 or a processed product thereof to a ketone in the presence of reduced form of nicotinamide adenine dinucleotide to generate an alcohol, and recovering the generated alcohol.

19. (Withdrawn) A method for producing an alcohol, the method comprising the steps of:

providing a microorganism producing the (R)-2,3-butanediol dehydrogenase of claim 1 or a processed product thereof;

reacting the (R)-2,3-butanediol dehydrogenase produced from the microorganism to a ketone in the presence of reduced form of nicotinamide adenine dinucleotide to generate an alcohol, and

recovering the generated alcohol.

20. (Withdrawn) The method of claim 19, wherein the microorganism is the transformant of claim 11.

21. (Withdrawn) The method of claim 17, wherein the ketone is 2,3-butanedione and the alcohol is (2R,3R)-2,3-butanediol.

22. (Withdrawn) The method of claim 18, wherein the ketone is 2,3-butanedione and the alcohol is (2R,3R)-2,3-butanediol.

23. (Withdrawn) The method of claim 19, wherein the ketone is 2,3-butanedione and the alcohol is (2R,3R)-2,3-butanediol.

24. (New) An isolated polypeptide the amino acid sequence of which comprises a sequence at least 70% percent identical to the amino acid sequence of SEQ ID NO: 2, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase that:

- (a) produces (R)-acetoin by acting on (2R,3R)-2,3-butanediol using nicotinamide adenine dinucleotide as a coenzyme and produces (2R,3R)-2,3-butanediol by reducing 2,3-butanedione using a reduced form of nicotinamide adenine dinucleotide as a coenzyme; and
- (b) uses nicotinamide adenine dinucleotide as a coenzyme in an oxidation reaction;
- (c) uses a reduced form of nicotinamide adenine dinucleotide as a coenzyme in a reduction reaction; and
- (d) preferentially oxidizes a hydroxyl group of 2,3-butanediol in (R) configuration.

25. (New) The isolated polypeptide of claim 24, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

26. (New) An isolated polypeptide encoded by a polynucleotide that is at least 80% identical to a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase that:

- (a) produces (R)-acetoin by acting on (2R,3R)-2,3-butanediol using nicotinamide adenine dinucleotide as a coenzyme and produces (2R,3R)-2,3-butanediol by reducing 2,3-butanedione using a reduced form of nicotinamide adenine dinucleotide as a coenzyme; and
- (b) uses nicotinamide adenine dinucleotide as a coenzyme in an oxidation reaction;
- (c) uses a reduced form of nicotinamide adenine dinucleotide as a coenzyme in a reduction reaction;

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(d) preferentially oxidizes a hydroxyl group of 2,3-butanediol in (R) configuration.

27. (New) The isolated polypeptide of claim 26, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

28. (New) An isolated polypeptide, wherein the polypeptide is an (R)-2,3-butanediol dehydrogenase that:

(a) produces (R)-acetoin by acting on (2R,3R)-2,3-butanediol using nicotinamide adenine dinucleotide as a coenzyme and produces (2R,3R)-2,3-butanediol by reducing 2,3-butanedione using reduced form of nicotinamide adenine dinucleotide as a coenzyme;

(b) uses nicotinamide adenine dinucleotide as a coenzyme in an oxidation reaction;

(c) uses a reduced form of nicotinamide adenine dinucleotide as a coenzyme in a reduction reaction;

(d) preferentially oxidizes a hydroxyl group of 2,3-butanediol in (R) configuration;

(e) has a specific activity of about 100 U/mg or higher when purified;

(f) has an optimal pH of 10 for a glycerol oxidation reaction; and

(g) has a molecular weight of about 36,000 Da when determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and about 76,000 Da when determined by gel filtration.

29. (New) The isolated polypeptide of claim 28, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

30. (New) The isolated polypeptide of claim 29, wherein the microorganism is *Pichia angusta*.

31. (New) The isolated polypeptide of claim 24, wherein the polypeptide comprises a sequence at least 80% percent identical to the amino acid sequence of SEQ ID NO:2.

32. (New) The isolated polypeptide of claim 24, wherein the polypeptide comprises a sequence at least 90% percent identical to the amino acid sequence of SEQ ID NO:2.

33. (New) The isolated polypeptide of claim 24, wherein the polypeptide comprises a sequence at least 95% percent identical to the amino acid sequence of SEQ ID NO:2.

34. (New) An isolated polypeptide the amino acid sequence of which consists of SEQ ID NO:2.

35. (New) An isolated polypeptide the amino acid sequence of which comprises SEQ ID NO:2.

36. (New) An isolated polypeptide the amino acid sequence of which comprises SEQ ID NO:2 with 0 to 50 conservative amino acid substitutions, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase.

37. (New) The isolated polypeptide of claim 36, wherein the amino acid sequence comprises 0 to 30 conservative amino acid substitutions.

38. (New) The isolated polypeptide of claim 36, wherein the amino acid sequence comprises 0 to 10 conservative amino acid substitutions.

39. (New) An isolated polypeptide the amino acid sequence of which comprises SEQ ID NO:5, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase.

40. (New) The isolated polypeptide of claim 39, wherein the dehydrogenase has a specific activity of about 100 U/mg or higher when purified.

41. (New) The isolated polypeptide of claim 40, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

42. (New) An isolated polypeptide the amino acid sequence of which comprises SEQ ID NO:4, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase.

43. (New) The isolated polypeptide of claim 42, wherein the dehydrogenase has a specific activity of about 100 U/mg or higher when purified.

44. (New) The isolated polypeptide of claim 42, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

45. (New) An isolated polypeptide the amino acid sequence of which comprises SEQ ID NO:3, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase.

46. (New) The isolated polypeptide of claim 45, wherein the dehydrogenase has a specific activity of about 100 U/mg or higher.

47. (New) The isolated polypeptide of claim 45, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

Applicant : Hiroaki Yamamoto et al.
Serial No. : 10/020,674
Filed : October 30, 2001
Page : 10 of 20

Attorney's Docket No.: 14875-092001 / D1-A0009-US

48. (New) An isolated polypeptide the amino acid sequence of which comprises SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5, wherein the polypeptide is a (R)-2,3-butanediol dehydrogenase.

49. (New) The isolated polypeptide of claim 48, wherein the dehydrogenase has a specific activity of about 100 U/mg or higher when purified.

50. (New) The isolated polypeptide of claim 48, wherein the dehydrogenase has the sequence of an enzyme naturally produced by a microorganism belonging to the genus *Pichia*.

Applicant : Hiroaki Yamamoto et al.
Serial No. : 10/020,674
Filed : October 30, 2001
Page : 11 of 20

Attorney's Docket No.: 14875-092001 / D1-A0009-US

Attachments Following Last Page of this Amendment:

1. Appendix A is a copy of Bowie, et al, (*Science* 247:1306)
2. Appendix B, part 1, provides a comparison between SEQ ID NO:2 of the present application and amino acid sequences set forth in the cited references of D1 and D2.
3. Appendix B, part 2, provides a sequence comparison between SEQ ID NO:1 of the present application and SEQ ID NO:1 of co-pending application No. 10/147,003 ('003) (represented as "D3" in the appendices).
4. Appendix B, part 3, provides a sequence comparison between SEQ ID NO:2 of the present application and SEQ ID NO:2 of co-pending application No. 10/147,003 ('003) (represented as "D3" in the appendices).
3. A certified copy of a translation of the Japanese patent application to which the pending application claims priority. The filing date of the priority document is October 31, 2000.